Journal of Surgical Oncology 61:290–294 (1996)

Comparison Between the In Vitro Intrinsic Radiation Sensitivity of Human Soft Tissue Sarcoma and Breast Cancer Cell Lines

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The purpose of this study is to evaluate the radiation sensitivity of human soft tissue sarcoma cell lines in vitro and to compare with that of human breast carcinoma and glioblastoma cell lines. The intrinsic radiation sensitivity parameters of seven human soft tissue sarcomas and eight breast carcinoma cell lines were investigated in vitro by clonogenic assays for single-dose irradiation under aerobic conditions on cells in exponential phase of growth. The results for sarcoma cell lines showed that the mean surviving fraction at 2 Gy (SF2) was 0.39 (SD ± 0.09) with a range of 0.24 to 0.53, and the average mean inactivation dose (MID) was 1.92 (SD ± 0.35) range from 1.36 Gy to 2.49 Gy. These values were not different from that of breast cell lines examined concurrently and using the same experimental methods (mean SF2 0.38, SD ± 0.09; MID 1.9 Gy, SD ± 0.37). However, radiobiological parameters of nine karyotyped human malignant glioma cell lines determined earlier in this laboratory were significantly higher (mean SF2 0.50 ± 0.14; mean MID 2.61 ± 0.60). In conclusion, the data presented here do not support the view that cells of sarcomas show unusual radiation resistance. To the extent that the in vitro determined cellular radiation sensitivity reflects the tumor response in vivo, the success rate for radiation applied against sarcoma and breast carcinoma of comparable size could be similar.

KEY WORDS: **intrinsic radiation sensitivity, SF2, mean inactivation dose, soft tissue sarcomas, breast carcinoma cell lines**

INTRODUCTION

The probability of local control of soft tissue sarcomas (STS) following combined treatment, i.e., limb-saving surgery and perioperative radiation therapy, is >85%, which compares favorably with the results achieved by surgery alone [1–5]. The basis for the success in local control of high-grade STS by limb salvage procedures almost certainly includes high efficiency of radiation to kill tumor cells that have extended beyond the gross lesion, better understanding of surgical pathology of the primary tumor, and more strictly defined intraoperative margins.

Almost all data relevant to these factors are based exclusively on the results of clinical experience. Little information is available on the radiosensitivity of soft tissue sarcoma cell lines. There had been a strongly entrenched clinical notion that the STS, other than extraosseous Ewing's sarcoma, were radiation-resistant tumors. The clinical success of radiation combined with surgery for these tumors raises questions regarding the radiation

Accepted for publication November 23, 1995.

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resistance of cells of sarcomas relative to that of cells of epithelial tumors, which are often treated successfully.

The basic parameter in assessing the clinical radiation resistance is the intrinsic radiation sensitivity. By this term, we refer to the radiation sensitivity of STS and breast cancer cell lines when irradiated as individual cells cultured in vitro under optimal metabolic conditions and cell survival scored on the basis of colony formation. The surviving fractions at 2 Gy (SF2) and the mean inactivation dose (MID) are considered to be the most clinically relevant parameters of radiation sensitivity of colony formation assays [6–11] as they relate to the sensitivity to radiation in the dose range most often applied clinically. Two laboratories have published data on the intrinsic radiation sensitivity of human sarcoma cell lines. Weichselbaum et al. [12–14] described the results on cell lines established from both bone sarcomas and STS; the mean SF2 was 0.27 and 0.34, respectively. Stuschke et al. [15,16] tested five STS cell lines as multicellular spheroids or as exponential phase cells; the mean values of SF2 were 0.43 (range 0.31–0.68) and 0.24 (range 0.19–0.38) for the two systems, respectively. For head and neck squamous cell carcinomas, Brock et al. [17] published the mean SF2 of 0.33 ± 0.15 derived from 72 tumor cultures, using population growth assay. Those results and other data indicate that the radiation sensitivity of sarcoma cell lines is comparable to that of epithelial tumor cells [9,13,18].

Studies exploring the radiosensitivity of human sarcoma cell lines characterized by histological grade, sites of biopsy of tissue taken for culture, and DNA ploidy may be valuable to the future application of external or interstitial irradiation and its timing in the combined treatment of these tumors. This report is an account of the initial phase of research designed to assess radiation response characteristics of human STS based on experiments performed both in vitro and in vivo. Here, the results of in vitro assays of the radiation sensitivity of seven cell lines derived from human STS and eight cell lines derived from human breast cancer (HBC) are described in terms SF2 and MID. These results and values of other radiobiological parameters: D_0 (calculated from the slope of the distal part of the survival curve and is defined as the dose required to reduce the fraction of surviving cells to 37% of its previous value), and n (the extrapolation number and is a mesure of the width of the shoulder of the survival curve), were compared to those of nine karyotyped glioblastoma cell lines reported previously from this laboratory.

MATERIALS AND METHODS

Tumor Cell Lines

Six human sarcoma cell lines employed in this study were established by J. A. Fletcher, and the seventh was provided by Mr. William Dahlberg, Harvard School of Public Health (derived from a primary tumor of a patient treated at Massachusetts General Hospital). The anatomic site of origin, site of biopsy, and the histology of each cell line are summarized in Table I. Breast cancer cell lines used in this work were obtained as frozen stock from the American Type Culture Collection (ATCC, Rockville, MD).

TABLE I. Human Soft Tissue Sarcoma Cell Lines Characteristics

Cell lines	Histology[a]	Biopsy site
STS 26T	MSCHw, G3	primary
ST 89-176	RMSa, G3	local recurrence
ST 90-244	RMSa, G3	primary
ST 88-14	MSchw, G2	primary
ST 90-208	LMSa, G3	primary
ST 91-247	EndSa, G1	lung met.[b]
ST 91-248	EndSa, G1	lung met.[b]

[a]Abbreviations: RMSa: embryonal rhabdomyosarcoma; MSCHw: malignant schwannoma; LMSa: leiomyosarcoma; EndSa: endometrial sarcoma; G: grade.

[b]Two cell lines derived from two different lung metastases from the same patient.

The cell lines were maintained in Dulbecco's modified Eagle medium with 20% heat inactivated fetal bovine serum for sarcoma or 10% serum for breast cancer cell lines, and antibiotics (0.05 mg penicillin/ml, 0.05 mg streptomycin/ml and 0.1 mg neomycin sulfate/ml) at 37°C with water vapor and in an atmosphere of 5% CO2 in air. For STS cell lines, passage levels 6–46 were used for experiments (80% of the assays were performed on cell lines older than 12th passage). All breast cell lines were investigated in high passages as they were obtained from ATCC as established lines.

Radiation Cell Survival Assays

All cell lines were studied in exponential phase. For the assays, single-cell suspensions of exponentially growing cells were obtained by disaggregation of cells attached to plastic by 0.05% trypsin; then, the cells were plated on 25 cm^2 plastic flasks in numbers appropriate for colony counting. Feeder cells were added to the viable cells in lower dose levels to bring the total cell number to ~40,000. Feeder cells (treated by 40 Gy) were cells of the same line as the studied cells. At 17–24 hr after plating, cells in monolayers were irradiated using 250 kVp X-rays (~0.4 mm Cu HVL, 1.6 Gy/min, and 50 cm target cell distance). Irradiations were performed with the flasks placed on a rotating lucite block at room temperature, 17–20°C. Seven dose levels from 1 to 11 Gy, were used. Six replicate flasks were plated for each dose level. The flasks were left undisturbed at 37°C in an atmosphere of 5% CO2 in aerobic conditions for 17–28 days. The flasks were then gently washed and the cultures fixed with methanol, stained using crystal violet and the colonies were counted. Only colonies of >50 cells were scored as survivors. Complete and independent assays

were performed three or four times for each cell line. The results presented in this report were obtained during a 4-month period. Plating efficiency (PE) experiments were performed concurrently with each colony formation assay using the same techniques as described above.

The increase in cell number between inoculation and irradiation (multiplicity) was investigated. The fractional increase in cell number during the 17–24 hours after plating was 1.08 ± 0.07 and a coefficient of variation (CV) of 6%. Therefore, the potential error due to cell multiplicity at the time of irradiation was judged as non-significant for the purpose of comparison of the radiation sensitivity of the different human tumor cell lines in this study.

Data Analysis

Analysis of cell survival curve data was performed on the pooled data from all experiments for each cell line (all flasks for each dose level) and based on specially created macro-files for Microsoft Excel 3.0. For the calculation of D_0 and n, lnSF (SF = <0.1) vs. dose was fitted by simple linear regression (by use of a software package StatWorks, Cricket Software, Philadelphia, PA). All data points were weighted equally. The SF2 and MID were calculated using the linear quadratic model and by the published method [7,10].

RESULTS

Sarcoma Cell Line Characteristics

Two cell lines were derived from malignant schwannomas of the left scapular region and retroperitoneum, two from adult embryonal rhabdomyosarcoma of the chest wall soft tissue and paravertebral region, two from endometrial stroma sarcomas, and one from a leiomyosarcoma of the chest wall (Table I). The pathology reports revealed that five cell lines were derived from high (G3) or intermediate (G2) grade sarcomas. However, the two cell lines from low grade sarcomas (ST91-247 and ST90-248) on which radiation cell survival assays were successfully performed were obtained from two different lung metastases, whereas we failed to performed the assays (lack of forming colony) on the cell line derived from the primary tumor, a low grade endometrial sarcoma, of the same patient.

Survival Curve Parameters

Soft tissue sarcoma cell lines. The values for survival curve parameters for soft tissue are shown in Table II. For sarcoma cell lines, the plating efficiency varied between 1.2% and 25%. The mean SF2 was 0.39 ± 0.09 with a range of 0.24 to 0.53, and a CV of 24%. The mean MID was 1.92 ± 0.35 Gy ranging from 1.36 to 2.49, and a CV of 18%. The slope of plots of cumulative frequency for values of these parameters (Fig. 1) indicate a relatively narrow range of intrinsic sensitivity for all sarcoma cell

TABLE II. Human Soft Tissue Sarcoma Cell Lines Survival Curve Parameters

Cell lines	PE[a] (%)	D0 (Gy)	n	SF2	MID (Gy)
STS 26T	13.9	1.06	4.53	0.38	1.88
ST 89-176	22.6	0.91	4.95	0.36	1.77
ST 90-244	24.8	1.39	3.44	0.53	2.48
ST 88-14	10	1.03	1.36	0.24	1.36
ST 90-208	9.5	1.12	5.94	0.47	2.21
ST 91-247	17.7	1.4	1.33	0.37	1.85
ST 91-248	1.2	1.52	3.42	0.35	1.9
Mean	14.2	1.20	3.57	0.39	1.92
SD[b]	8.21	0.23	1.75	0.09	0.35
CV[c]	58%	19%	49%	24%	18%

[a]Plating efficiency.
[b]Standard deviation.
[c]Coefficient of variation.

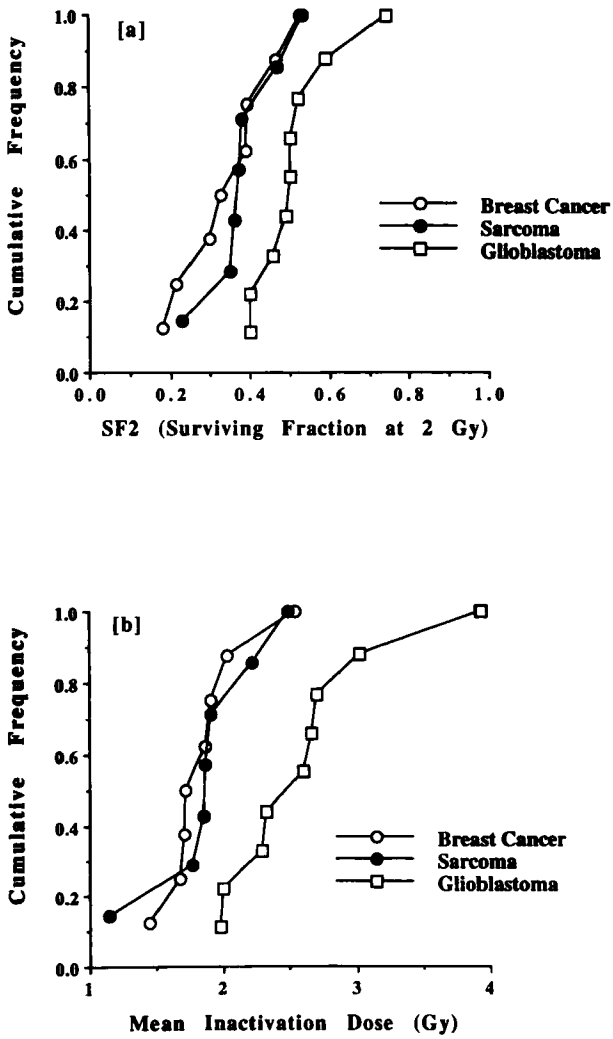

Fig. 1. Cumulative frequency of surviving fraction at 2 Gy (**a**) and the mean inactivation dose (**b**) of soft tissue sarcoma, breast cancer, and malignant glioma cell lines.

TABLE III. Human Breast Cancer Cell Lines Survival Curve Parameters

Cell lines (ATCC code)	PE[a] (%)	D0 (Gy)	n	SF2	MID (Gy)
BT-474 (HTB 20)	29.6	1.08	6.30	0.46	2.16
MCF-7 (HTB 22)	23.7	0.83	14.2	0.40	1.89
MDAMB-134VI (HTB 23)	61.6	1.69	2.24	0.54	2.62
MDAMB-157 (HTB 24)	28.3	1.07	4.43	0.36	1.79
MDAMB-175VII (HTB 25)	22.1	1.10	1.60	0.23	1.35
MDAMB-231 (HTB 26)	26.8	1.03	5.04	0.41	1.95
MDAMB-361 (HTB 27)	9.5	1.00	4.40	0.34	1.70
SK-BR-3 (HTB 30)	31.2	1.36	2.08	0.31	1.68
Mean	14.2	1.15	5.02	0.3	1.89
SD[b]	8.21	0.26	4.05	0.09	0.38
CV[c]	58%	23%	81%	25%	20%

[a]Plating efficiency.
[b]Standard deviation.
[c]Coefficient of variation.

lines, despite their clearly different histological subtypes. Table II also shows the D_0 and n values calculated from the survival curves.

Breast cancer cell lines. The values for survival curve parameters for breast carcinomas are shown in Table III. The plating efficiency (PE) varied between 9.5 and 61.6%, with a mean value of 29.1% + 14.8, and a CV of 51%. Survival at 2 Gy ranged from 0.23 to 0.54 with a mean of 0.38 + 0.09 (CV 25%). The MID ranged from 1.35 to 2.62, with a mean value of 1.9 ± 0.37, and a CV of 20%.

Soft Tissue Sarcoma vs. Breast Carcinoma and Malignant Glioblastoma Cell Lines

Figure 1 shows the cumulative frequency of SF2 and MID for human soft tissue sarcoma versus breast carcinoma and glioblastoma cell lines. No differences were found between STS and breast carcinoma cell lines in relation to these parameters. However, using the same end point of all the assays (performed in the same lab), significantly higher values of SF2 for glioblastomas were found for SF2 (mean 0.51 ± 0.10; $P = 0.025$), and for MID (mean 2.63 ± 0.60; $P = 0.018$).

DISCUSSION

The predictive power of SF2 or MID for the response to radiation treatment of human tissue has been a subject of intensive investigation in numerous laboratories for several years. The parameters of radiation sensitivity D_0 and n have been shown to be poor predictors to radiation response. However, radiation sensitivity described in terms of the parameters SF2 or MID are often accepted as correlating reasonably well with the response patterns of the major histological types of tumors [6,10]. These parameters are determined primarily by the initial part of the survival curve, which appears to be the most clinically relevant part of the curve, as radiation is usually administered in small dose fractions 1.5–2.5 Gy [6–9,11,19].

In this study, all sarcoma cell lines were karyotyped using methods described previously [20]. Each cell line had at least three clonal chromosome aberrations, and these aberrations were identical to those characterized in the fresh tumor specimen, from which the cell lines were derived originally (data not shown).

The findings of this present investigation demonstrate that the inherent radiation sensitivity of the cell lines derived from sarcomas of soft tissue and carcinomas of the breast are not different, and both of them are significantly more sensitive than glioblastoma cell lines. This is based upon 15 cell lines tested (7 sarcoma and 8 breast tumor lines) compared to nine glioblastoma cell lines. Our mean SF2 value of 0.39 is comparable with that of previous studies by Weichselbaum et al. [12] and Stuschke et al. [16], who found average values of 0.34 and 0.24, respectively.

That observation is in accordance with the clinical experience, which yields local control rates of >90% for both carcinomas of the breast and sarcomas of the soft tissue treated by radiation and surgery even though the STS are much larger in an average diameter [5,21]. These are not manifestly different from that reported for locally advanced carcinomas of the breast treated by radiation alone with estimated local recurrence rates varying widely, from 41% to 75% [22,23]. Local control rates of 30–50% for STS treated by radiation alone have been reported by several centers [24–27].

A complicating factor in assessing the predictive value of the in vitro measures of radiosensitivity is that modern treatment of solid tumors is based on a multidisciplinary approach and irradiation is only one of the modalities employed. The tumor cells in vitro are tested under near optimal metabolic conditions, whereas cells in tumors in patients live under widely differing pathophysiological conditions. Some of these conditions drastically effect the cellular sensitivity to radiation. Thus a local treatment failure does not directly connect with experimentally determinant values of inherent radiosensitivity.

Nevertheless, these data show that the in vitro radiation sensitivity of these cell lines indicate that soft tissue sarcoma and breast tumor cell lines are similar. To the extent that the in vitro determined cellular raditiation sensitivity reflects the tumor response in vivo [10,19,28], the success rate for radiation applied against sarcoma and breast carcinoma of comparable size could be similar.

ACKNOWLEDGMENTS

This work was supported in part by grant CA13311 awarded by the National Cancer Institute, National Institute of Health, Department of Health and Human Services. W.R. was a visiting surgeon at the Edwin L. Steele Laboratory of Radiation Biology. Supported in part by the

National Cancer Institute and Cancer Research Campaign under the EORTC/NCI Exchange Program.

REFERENCES

1. Emrich LJ, Ruka W, Driscoll DL, Karakousis CP: The effect of local recurrence on survival time in adult high-grade soft tissue sarcomas. J Clin Epidemiol 42:105–110, 1989.
2. Gerner RE, Moore G, Pickren J: Soft tissue sarcoma. Ann Surg 181:803–808, 1975.
3. Heise HW, Myers MH, Russell WO, et al.: Recurrence-free survival time for surgically treated soft tissue sarcoma patients. Cancer 57:172–177, 1986.
4. Shiu MH, Castro B, Hajdu SI, Fortner JG: Surgical treatment of 297 soft tissue sarcomas of the lower extremity. Ann Surg 182:597–602, 1975.
5. Suit HD, Mankin HJ, Wood WC, et al.: Treatment of patients with stage Mo soft tissue sarcoma. J Clin Oncol 6:854–862, 1988.
6. Deacon J, Peckham MJ, Steel G: The radioresponse of human tumors and the initial slope of the cell survival curve. Radiother Oncol 2:317–323, 1984.
7. Fertil B, Dertinger H, Courdi A, Malaise EP: Mean inactivation dose: a useful concept for intercomparison of human cell survival curves. Radiat Res 99:73–84, 1984.
8. Fertil B, Malaise EP: Inherent cellular radiosensitivity as a basic concept for human tumor radiotherapy. Int J Radiat Oncol Biol Phys 7:621–629, 1981.
9. Fertil B, Malaise EP: Intrinsic radiosensitivity of human cell lines is correlated with radioresponsiveness of human tumors: Analysis of 101 published survival curves. Int J Radiat Oncol Biol Phys 11:1699–1707, 1985.
10. Malaise EP, Fertil B, Chavaudra N, Guichard M: Distribution of radiation sensitivities for human tumor cells of specific histological types: Comparison of in vitro to in vivo data. Int J Radiat Oncol Biol Phys 12:617–624, 1986.
11. Malaise EP, Fertil B, Deschavanne P, et al.: Initial slope of radiation survival curves is characteristic of the origin of primary and established cultures of human tumor cells and fibroblasts. Radiat Res 111:319–333, 1987.
12. Weichselbaum RR, Beckett MA, Simon MA, et al.: In vitro radiobiological parameters of human sarcoma cell lines. Int J Radiat Oncol Biol Phys 15:937–942, 1988.
13. Weichselbaum RR, Beckett MA, Vijayakumar S, et al.: Radiobiological characterization of head and neck and sarcoma cells derived from patients prior to radiotherapy. Int J Radiat Oncol Biol Phys 19:313–319, 1990.
14. Weichselbaum RR, Rotmensch J, Ahmed-Swan S, Beckett MA: Radiobiological characterization of 53 human tumor cell lines. Int J Radiat Biol 56:553–560, 1989.
15. Stuschke M, Budach V, Budach W, et al.: Multicellular spheroids from human soft tissue sarcomas: Radiocurability and dose fractionation effect. Int J Radiat Biol 56:549–552, 1989.
16. Stuschke M, Budach V, Klaes W, Sack H: Radiosensitivity, repair capacity, and stem cell fraction in human soft tissue tumors an in vitro study using multicellular spheroids and the colony assay. Int J Radiat Oncol Biol Phys 23:69–80, 1992.
17. Brock WA, Baker FL, Wike J, et al.: Cellular radiosensitivity of primary head and neck squamous cell carcinomas and local tumor control. Int J Radiat Oncol Biol Phys 18:1283–1286, 1990.
18. Girinsky T, Lubin R, Pignon JP, et al.: Predictive value of in vitro radiosensitivity parameters in head and neck cancers and cervical carcinomas: Preliminary correlations with control and overall survival. Int J Radiat Oncol Biol Phys 25:3–7, 1993.
19. Suit H, Baumann M, Skates S, Convery K: Clinical interest in determination of cellular radiation sensitivity. Int J Radiat Biol 56:725–737, 1989.
20. Fletcher JA, Kozakewich HP, Hoffer FA, et al.: Diagnostic relevance of clonal cytogenetic aberrations in malignant soft-tissue tumors. N Engl J Med 324:436–443, 1991.
21. Fisher B, Anderson SI, Fisher ER, et al.: Significance of ipsilateral breast tumour recurrence after lumpectomy. Lancet 338:327–331, 1991.
22. Arriagada R, Mouriesse H, Sarrazin D, et al.: Radiotherapy alone in breast cancer. I. Analysis of tumor parameters, tumor dose and local control: The experience of the Gustave-Roussy Institute and the Princess Margaret Hospital. Int J Radiat Oncol Biol Phys 11:1751–1575, 1985.
23. Borger JH, van Tienhoven G, Passchier DH, et al.: Primary radiotherapy of breast cancer: Treatment results in locally advanced breast cancer and in operable patients selected by positive axillary apex biopsy. Radiother Oncol 25:1–11, 1992.
24. McNeer GP, Cantin J, Chu F, Nickson J: Effectiveness of radiation therapy in the management of sarcoma of the soft somatic tissues. Cancer 22:391–397, 1968.
25. Perry H, Chu F: Radiation therapy in the palliative management of soft tissue sarcomas. Cancer 15:179–183, 1962.
26. Tepper JE, Suit HD: Radiation therapy alone for sarcoma of soft tissue. Cancer 56:475–479, 1985.
27. Windeyer B, Dische S, Mansfield CM: The place of radiotherapy in the management of fibrosarcoma of the soft tissues. Clin Radiol 17:32–40, 1966.
28. Brock WA: Cellular radiosensitivity as predictors of treatment outcome: Where do we stand? Int J Radiat Oncol Biol Phys 25:147–148, 1993.